REMARKS

Claims 1, 4-7, 13 and 23-25 are pending. Claims 1, 4-7, 13 and 25 have been amended. Claims 2, 3, 8-12, 14-22 and 26-29 are canceled. No new matter is added. Support for the amending language "transcription and/or translation" may be found in the specification at paragraph 33, among others. Support for the amending language "membrane vesicles containing respiratory chain components" may be found in the specification at paragraph 36. Support for the amending language "oxidative phosphorylation, which is sensitive to electron transport chain inhibitors" may be found in the specification at paragraphs 61-62.

Claims 1, 3-7 13-25 have been rejected under 35 U.S.C. 112, first paragraph as failing to comply with the written description requirement. The Office Action states that while the specification provides general information on producing mRNA and polypeptides in an in vitro cell-free expression system, there is no disclosure of the components or conditions necessary for the production of other types of polynucleotides, such as DNA.

Without conceding to the correctness of the rejection, Applicants have amended Claim 1 to recite specifically that the reaction is a transcription and/or translation reaction. Applicants have specifically exemplified such transcription and translation reactions, and have specifically exemplified coupled transcription and translation reactions.

Applicants respectfully submit that a representative number of reaction chemistries for the transcription of mRNA and translation of polypeptides are provided, and have set forth working examples in view of which a skilled artisan could practice the claimed invention. In view of the Applicants' teachings of both the various reaction chemistries that can be used, especially when coupled with the skilled artisan's common knowledge of how to generally synthesize polynucleotides and polypeptides in a cell free system, Applicants contend that one of skill in the art in view of Applicants' disclosure would be able to perform the synthesis as set forth in the claims.

The Office Action further states that while the specification describes the reaction mix prepared from E. coli grown under specific conditions, which result in activated oxidative phosphorylation, there is no disclosure of other types of reaction mixes having this property.

Applicants note that the present claims are directed to the use of extracts from bacterial cells grown in glucose and phosphate containing medium comprising components of polypeptide and/or mRNA synthesis machinery; a template for transcription of said mRNA and/or translation of said polypeptide; monomers for synthesis of said mRNA and/or

polypeptides; and co-factors, enzymes and other reagents necessary for said transcription and/or translation.

Applicants respectfully submit that cell-free extracts are known in the art from various bacteria, including, for example, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Methanococcus vannielii, Methanobacterium formicicum* and *Methanosarcina barker,* as described in the attached articles and abstracts. One of skill in the art is readily apprised of methods for growing many bacterial species, and of deriving suitable extracts from such species. In view of Applicants teachings, one of skill in the art is readily apprised of the means to practice the claimed invention, and no undue experimentation is required.

The rejection of Claims 22, 27 and 28 under 35 U.S.C. 112, second paragraph are made moot by the cancellation of the claims. Claim 25 has been amended to correct the antecedent support. Withdrawal of the rejection is requested.

The rejection of Claims 22 and 29 for double patenting is made moot by cancellation of the claims.

Claims 1, 3-7, 13, 22-25 and 27-29 have been rejected under 35 U.S.C. 103(a) as being unpatentable in view of Baranov *et al.* (1993) and Chen *et al.* in view of Yoshida *et al.*; Dorner *et al.*, Shimizu *et al.*, or Raney *et al.*

Applicants respectfully submit that the presently claimed invention is not made obvious by the cited art. Applicants respectfully submit that Baranov *et al.* fail to teach the activation of oxidative phosphorylation, and fail to demonstrate an ability to synthesis polypeptides and/or mRNA in the absence of a high energy phosphate source.

Applicants respectfully submit that the demonstration of oxidative phosphorylation is shown in the examples of the present application through a showing of polypeptide yield in the presence of absence of oxygen, and how inhibitors of the electron transport chain affect synthesis.

Figure 6 shows protein synthesis results of polypeptide under three separate conditions, with or without 2-heptyl-4-hydroxyquinoline-N-oxide (HQNO). HQNO inhibits the electron transport chain part of oxidative phosphorylation. The head spaces of the batch reactors were filled with either oxygen or argon, an inert gas. If oxidative phosphorylation is active, one would expect that the protein synthesis yield should decrease upon the addition of the inhibitor of

oxidative phosphorylation, HQNO, in the cases where oxygen is present. Furthermore, protein synthesis should be unaffected upon the addition of HQNO in the cases where argon is present and oxygen is not available.

The data in figure 6 support the conclusion that oxidative phosphorylation has been activated by the new environment. In the presence of oxygen, synthesis using the methods set forth in the present claims, with or without pyruvate, substantially decreases after HQNO addition and is unaffected by HQNO in the argon cases. Similar studies have been carried out with the following inhibitors of oxidative phosphorylation: 2,4-dinitrophenol, cyanide, azide, thenoyltrifluoroacetone, and carbonyl-cyanide-m-chlorophenylhydrazone. In all cases, the results support our conclusion that the new system has activated oxidative phosphorylation. It is important to note that protein synthesis yields in the PANOx system are unaffected by these same inhibitors. Claim 1 has been amended to recite that the oxidative phosphorylation is sensitive to such inhibitors, in contrast to methods such as the PANOx system.

The activation of oxidative phosphorylation provides unexpected benefits, which could not have been predicted from the cited art. The methods as set forth in the present claims provide for high levels of protein synthesis in the absence of an exogenous high energy phosphate source. For example, in Figure 2 it is shown that a yield of 700 μg/ml can be achieved in the absence of a high energy phosphate source, using the glycolytic intermediate pyruvate as source of energy. Even in the absence of pyruvate, the methods as set forth in the present claims generated greater than 300 μg/ml polypeptide.

As demonstrated in Figure 6, it is the ability to activate oxidative phosphorylation that provides for such an increase in yield. Under conditions as set forth in the present claims, greater than 600 μg/ml of protein is produced in a batch reaction in the absence of a high energy phosphate source where oxygen is present (and thus where oxidative phosphorylation is activated), while in the presence of an electron transport chain inhibitor the yield drops to less than 300 μg/ml; and in the presence of argon (where no oxygen is present for oxidative phosphorylation) the yield drops to less than 150 μg/ml.

The unexpectedly high yields of the present invention may be compared to those of Baranov *et al.* Baranov *et al.* required a high energy phosphate source for the reaction, 10 mM phosphoenol pyruvate. It should be noted that Applicants utilized a batch reaction, while Baranov *et al.* were feeding, in a 0.5 ml. reaction, 1.5 ml/hr over 24 hours, thus providing for feeding 36 mls., more than 70 times the initial reaction volume.

In spite of the presence of a high energy phosphate source, and in spite of providing the reaction with copious quantities of reagents during the feeding process, Baranov *et al.* were only able to obtain a total protein yield of 10 μg (presumably 20 μg/ml of starting reaction). Applicants are able to obtain 10 to 35 times greater yields (based on cell extract volume), using the presently claimed methods.

Applicants respectfully submit that such an enormous increase in production could not have been predicted by the prior art, and involves far more than routine experimentation. In particular, one of skill in the art could not have predicted the activation of oxidative phosphorylation, which is evidenced by sensitivity to specific inhibitors, and which provides for the unexpected increase in yield.

CONCLUSION

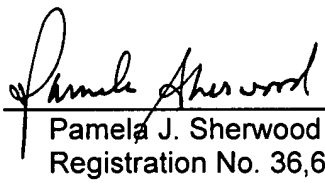
In view of the above amendments and remarks, Applicants respectfully submit that the presently claimed invention is not taught or suggested by the cited reference. Withdrawal of the rejections is requested.

Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-273.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: November 1, 2006

By: 
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